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(FILE 'HOME' ENTERED AT 14:21:21 ON 24 JUN 2003)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 14:22:14 ON 24 JUN 2003

SEA (SIALYLTRANSFERASE OR GALACTOSYLTRANSFERASE OR FUCOSYLTRANS

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27 FILE ADISCTI  
3 FILE ADISINSIGHT  
2 FILE ADISNEWS  
593 FILE AGRICOLA  
79 FILE ANABSTR  
45 FILE AQUASCI  
88 FILE BIOBUSINESS  
40 FILE BIOCOMMERCE  
7016 FILE BIOSIS  
805 FILE BIOTECHABS  
805 FILE BIOTECHDS  
4404 FILE BIOTECHNO  
1352 FILE CABA  
1883 FILE CANCERLIT  
9063 FILE CAPLUS  
194 FILE CEABA-VTB  
17 FILE CEN  
28 FILE CIN  
297 FILE CONFSCI  
6 FILE CROPU  
196 FILE DDFB  
113 FILE DDFU  
2771 FILE DGENE  
196 FILE DRUGB  
2 FILE DRUGNL  
134 FILE DRUGU  
1 FILE DRUGUPDATES  
68 FILE EMBAL  
7260 FILE EMBASE  
3230 FILE ESBIODASE  
170 FILE FEDRIP  
70 FILE FROSTI  
643 FILE FSTA  
5391 FILE GENBANK  
354 FILE IFIPAT  
3506 FILE JICST-EPLUS  
1 FILE KOSMET  
1831 FILE LIFESCI  
2 FILE MEDICONF  
6906 FILE MEDLINE  
9 FILE NIOSHTIC  
17 FILE NTIS  
5 FILE OCEAN  
6630 FILE PASCAL  
3 FILE PHAR  
1 FILE PHARMAML  
9 FILE PHIN  
69 FILE PROMT  
6572 FILE SCISEARCH  
1967 FILE TOXCENTER  
1507 FILE USPATFULL  
35 FILE USPAT2

2 FILE VETB  
3 FILE VETU  
420 FILE WPIDS  
420 FILE WPINDEX

L1 QUE (SIALYLTRANSFERASE OR GALACTOSYLTRANSFERASE OR FUCOSYLTRANS  
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FILE 'CAPLUS, EMBASE, BIOSIS, MEDLINE, PASCAL, SCISEARCH, BIOTECHNO,  
JICST-EPLUS, ESBIODASE, TOXCENTER, CANCERLIT, LIFESCI' ENTERED AT  
14:24:29 ON 24 JUN 2003

L2 1572 S L1 AND TRANSMEMBRANE  
L3 227 S L2 AND (DELET? OR DEVOID OR REMOV?)  
L4 76 DUP REM L3 (151 DUPLICATES REMOVED)  
L5 1 S L4 AND ASPERGILLUS

ACCESSION NUMBER: 1992:148892 CAPLUS

DOCUMENT NUMBER: 116:148892

TITLE: The signal for Golgi retention of bovine .beta.1,4-galactosyltransferase is in the transmembrane domain

AUTHOR(S): Teasdale, Rohan D.; D'Agostaro, Giacomo; Gleeson, Paul A.

CORPORATE SOURCE: Med. Sch., Monash Univ., Melbourne, 3181, Australia

SOURCE: Journal of Biological Chemistry (1992), 267(6), 4084-96

CODEN: JBCHA3; ISSN: 0021-9258

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The expression and localization of bovine .beta.1,4-galactosyltransferase (Gal T) was studied in mammalian cells transfected with Gal T cDNA constructs, and the role of N-terminal domains of Gal T in Golgi localization examd. Here it is demonstrated that the transmembrane (signal/anchor) domain of bovine Gal T contains a pos. Golgi retention signal. Bovine Gal T was characterized in transfected cells with antibovine Gal T antibodies, affinity-purified from a rabbit antiserum using a bacterial recombinant fusion protein. These affinity-purified antibodies recognized native bovine Gal T and showed min. cross-reactivity with Gal T from nonbovine sources. Bovine Gal T cDNA was expressed, as active enzyme, transiently in COS-1 cells and stably in murine L cells, and the product was shown to be localized to the Golgi complex by immunofluorescence using the polypeptide-specific antibodies. A low level of surface bovine Gal T was also detected in the transfected L cells by flow cytometry. The removal of 18 of the 24 amino acids from the cytoplasmic domain of bovine Gal T did not alter the Golgi localization of the product transiently expressed in COS-1 cells or stably expressed in L cells. Both the full-length bovine Gal T and the cytoplasmic domain deletion mutant were N-glycosylated in the transfected L cells, indicating both proteins have the correct Nin/Cout membrane orientation. Deletion of both the cytoplasmic and signal/anchor domains of bovine Gal T and incorporation of a cleavable signal sequence resulted in a truncated sol. bovine Gal T that was rapidly secreted (within 1 h) from transfected COS-1 cells. Replacement of the signal/anchor domain of bovine Gal T with the signal/anchor domain of the human transferrin receptor resulted in the transport of the hybrid mol. to the cell surface of transfected COS-1 cells. Furthermore, a hybrid construct contg. the signal/anchor domain of Gal T with ovalbumin was efficiently retained in the Golgi complex, whereas ovalbumin anchored to the membrane by the transferrin receptor signal/anchor was expressed at the cell surface of transfected COS-1 cells. Overall, these studies show that the hydrophobic, signal/anchor domain of Gal T is both necessary and sufficient for Golgi localization.

L4 ANSWER 68 OF 76 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1993:287208 BIOSIS

DOCUMENT NUMBER: PREV199345005333

TITLE: Study of the functional domains of beta-1-4  
**galactosyltransferase** through the expression of the  
**deletion** constructs of cDNA in Escherichia coli and  
mammalian cells: Disulfide bond between CYS 134 and CYS 247  
is required for folding and enzyme activity while the  
**transmembrane** domain is essential for stable  
expression in mammalian cells.

AUTHOR(S): Qasba, Pradman K.; Masibay, Arni S.; Boeggeman, Elizabeth  
E.; Balaji, Peety V.

CORPORATE SOURCE: Div. Cancer Inst., Natl. Inst. Health, Bethesda, MD 20892  
USA

SOURCE: Protein Engineering, (1993) Vol. 6, No. SUPPL., pp. 99.  
Meeting Info.: Winter Symposium on Advances in Gene  
Technology: Protein Engineering and Beyond Miami, Florida,  
USA 1993

ISSN: 0269-2139.

DOCUMENT TYPE: Conference

LANGUAGE: English

L4. ANSWER 64 OF 76 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 26

ACCESSION NUMBER: 1993:554766 CAPLUS

DOCUMENT NUMBER: 119:154766

TITLE: Mutational analysis of the Golgi retention signal of bovine .beta.-1,4-**galactosyltransferase**

AUTHOR(S): Masibay, Arni S.; Balaji, Petety V.; Boeggeman, Elizabeth E.; Qasba, Pradman K.

CORPORATE SOURCE: Lab. Math. Biol., Natl. Cancer Inst., Bethesda, MD, 20892, USA

SOURCE: Journal of Biological Chemistry (1993), 268(13), 9908-16

CODEN: JBCHA3; ISSN: 0021-9258

DOCUMENT TYPE: Journal

LANGUAGE: English

AB To examine the role of the N-terminal region of the 402-residue-long acetylglucosamine .beta.-1,4-**galactosyltransferase** (.beta.-1,4-GT), a series of mutants and chimeric cDNA were constructed by polymerase chain reaction and transiently expressed in COS-7 cells, the enzyme activities were measured, and the protein was localized in the cells by subcellular fractionation or indirect immunofluorescence microscopy. It was shown earlier that the **deletion** of the N-terminal cytoplasmic tail and **transmembrane** domain from .beta.-1,4-GT abolishes the stable expression of this protein in mammalian cells. Further **deletion** analyses of the N-terminal region showed that the 1st 21 amino acids of .beta.-1,4-GT are not essential for the stable prodn. of the protein and are consistently localized in the Golgi app. In addn., anal. of hybrid constructs showed that residues 1-25 of .alpha.-1,3-**galactosyltransferase** can functionally replace the .beta.-1,4-GT N-terminal domain (residues 1-43). This fusion protein also showed Golgi localization. On the other hand, the .alpha.-2,6-**sialyltransferase**/.beta.-1,4-GT fusion protein (.alpha.-2,6-ST/.beta.-1,4-GT) needed addnl. C-terminal sequences flanking the **transmembrane** domain of the .alpha.-2,6-ST for stability and Golgi localization. Substitution of Arg-24, Leu-25, Leu-26, and His-33 of the .beta.-1,4-GT **transmembrane** by Ile (pLFM) or substitution of Tyr by Ile at positions 40 and 41 coupled with the insertion of 4 Ile residues at position 43 (pLB) released the mutant proteins from the Golgi and was detected on the cell surface. The results showed that (1) the **transmembrane** domains of .beta.-1,4-GT, .alpha.-1,3-**galactosyltransferase**, and .alpha.-2,6-ST, along with its stem region, all play a role in Golgi targeting and participate in a common mechanism that allows the protein to be processed properly and not be degraded in vivo; (2) increasing the length of the **transmembrane** domain overrides the Golgi retention signal and directs the enzyme to the plasma membrane; and (3) the length of the hydrophobic region of the **transmembrane** domain of .beta.-1,4-GT is an important parameter but is not sufficient by itself for Golgi retention.

ACCESSION NUMBER: 1994:526296 CAPLUS

DOCUMENT NUMBER: 121:126296

TITLE: Expression of soluble active human .beta.1,4-  
**galactosyltransferase** in *Saccharomyces cerevisiae*

AUTHOR(S): Kleene, Ralf; Krezdorn, Christian H.; Watzele, Gabriele; Meyhack, Bernd; Herrmann, Guido F.; Wandrey, Christian; Berger, Eric G.

CORPORATE SOURCE: Physiol. Inst., Univ. Zurich, Zurich, CH-8057, Switz.

SOURCE: Biochemical and Biophysical Research Communications (1994), 201(1), 160-7

CODEN: BBRCA9; ISSN: 0006-291X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Sequences coding for the cytoplasmic and **transmembrane** domains were **removed** from the cDNA of the human Golgi resident membrane protein .beta.1,4 **galactosyltransferase** (galT). The remaining sequences coding for the stem and catalytic domains of this **glycosyltransferase** were fused to sequences coding for the yeast invertase signal sequence. The hybrid was inserted together with a constitutive yeast promoter and a terminator into an *Escherichia coli*/yeast shuttle vector. *Saccharomyces cerevisiae* strain BT150 transformed with this new expression vector expressed enzymically active sol. enzyme, whereas no activity was detectable in mock-transformed yeasts. The enzyme product was identified by HPLC anal. and shown to correspond to the expected product N-acetyllactosamine.